TRANSLOCATION IN RIBOSOMES BY ATTACHMENT–DETACHMENT OF ELONGATION FACTOR G WITHOUT GTP CLEAVAGE: EVIDENCE FROM A COLUMN-BOUND RIBOSOME SYSTEM

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1. Introduction

It is well known that translocation in the ribosome is induced by the elongation factor G (EF-G) with a conjugated GTP cleavage [1,2]. It is assumed that the energy of GTP cleavage is expended on mechanical work in the process of translocation. Two different manners can be considered by which EF-G with GTP might be involved in translocation: 1) GTP cleavage by itself directly drives translocation; then EF-G plays primarily the role of a protein carrier of GTP. 2) Translocation is driven by the attachment of EF-G with GTP to the ribosome and/or by the detachment of EF-G with GDP; then the role of GTP comes to that of an allosteric effector required for the attachment of EF-G to the ribosome while GTP cleavage leads to the release of EF-G. The present communication is devoted to the experimental choice from the two possible alternative ways of utilizing the energy of GTP cleavage for translocation.

Recently two contradictory reports appeared [3,4]. Kaziro's group reported that incubation of pre-translocated ribosomes with EF-G and a non-cleavable GTP analogue, guanylyl-methylene diphosphonate (GMPPCP), resulted in translocation [4]. Vazquez's group found that in an analogous system translocation did not take place with GMPPCP, but proceeded strictly in conjunction and stoichiometrically with GTP hydrolysis [3]. Unfortunately in both groups, the experiments for testing translocation were done directly in the complex incubation mixture with EF-G and GTP or GMPPCP, and not after performing the separate stages of adding EF-G and then its removing. This led to some uncertainty in interpretation of the data for the choice from the abovementioned alternatives.

In the present experiment a ribosomal system of translation of polyuridylic acid (polyU) covalently coupled by its 3′-end to a cellulose carrier (solid-phase translation system [5]) has been used. It has been shown that the passing of EF-G with GMPPCP through the column containing pre-translocated ribosomes on the cellulose-bound polyU induces the same translocation as in the case of passing of EF-G with GTP. Thus, GTP cleavage does not seem to be necessary for translocation; it is likely that translocation in ribosomes is induced by the successive attachment and detachment of EF-G.

2. Materials and methods

PolyU-cellulose resin was prepared by coupling the periodate-oxydized 3′-end of polyU with the hydrazide derivative of carboxymethylcellulose (CM-32, Whatman) as described earlier [5]. The capacity was 8.4 μg of polyU per mg of the dry resin.

Escherichia coli MRE-600 ribosomes washed four times by 1 M NH_4Cl with 10 mM $MgCl_2$ [6,7] were used.

Purified elongation factors $T_u T_s$ and G were prepared from *E. coli* MRE-600 mainly according to the procedures described by Kaziro et al. [8,9].

E. coli tRNA (Serva) was acylated enzymatically with [^{14}C]phenylalanine (513 mCi/mmol, Amersham) as described previously [10]; the final preparation contained 435 pmoles of [^{14}C]phenylalanyl-tRNA per mg of total tRNA.

The system of translation of the cellulose-bound polyU by ribosomes in the presence of [^{14}C]phenylalanyl-tRNA, total fraction of elongation factors and GTP was made in the standard buffer containing 10 mM Tris–HCl, 10 mM $MgCl_2$, 100 mM KCl and 1 mM dithiothreitol (DTT), $pH_{25°C}$ 7.1, at 25°C [5]. After 10 min incubation the system was placed in columns and stopped by washing off all the components not bound to the polyU-cellulose using the same standard buffer at 25°C. A set of four parallel columns was operated simultaneously each time.

To prepare the pre-translocation state of elongating ribosomes bound to the polyU-cellulose, the mixture of [^{14}C]phenylalanyl-tRNA, EF-T_uT_s and GTP in the same buffer was passed through the columns during 10 min at 25°C, and then the columns were again washed off from the free components by the standard buffer with 20 mM $MgCl_2$ at 25°C. Such columns were directly used for the experiments on translocation.

In the experiments on translocation EF-G and/or GTP or GMPPCP in the standard buffer with 10 mM $MgCl_2$ were passed through the columns for 5 min at 25°C, and then the columns were washed by the standard buffer with 20 mM $MgCl_2$ at 25°C. 1 mM puromycin solution in the buffer containing 10 mM Tris–HCl, 20 mM $MgCl_2$, 160 mM NH_4Cl and 1 mM DTT (pH 7.2) was then passed for 10 min at 25°C. The columns with the released [^{14}C]polyphenylalanyl-puromycin were washed by the standard buffer with 20 mM $MgCl_2$; the eluate contained about 30% of the released polyphenylalanyl-puromycin while the remaining polyphenylalanyl-puromycin was firmly adsorbed on the resin. The columns were further washed with the standard buffer without $MgCl_2$ to dissociate translating ribosomes and thus elute the [^{14}C]polyphenylalanyl-tRNA which did not react with puromycin; practically all the latter came out from the resin and served as a measure of the pre-translocation state of ribosomes in the column. The resin-sorbed [^{14}C]polyphenylalanyl-puromycin was eluted from the column by 0.5 N KOH in ethanol. The radioactivity of the polyphenylalanyl-puromycin contained in both the aqueous (buffer with Mg^{2+}) and ethanolic (KOH) eluates was summarized and taken as the measure of the post-translocation state of ribosomes in the column.

The radioactivity of the polymerized [^{14}C]phenylalanine in all the eluates was counted after precipitating the radioactive material with trichloroacetic acid, its following hydrolysis for 15 min at 90°C and depositing the precipitates onto nitrocellulose filters; measurements were done in the liquid scintillation spectrometer (Beckman LS-100C) using the standard toluene-PPO-POPOP system.

3. Results and discussion

The scheme of the experiment is presented in fig.1. The results are given in table 1. It is seen from

STAGES:	SOLID-PHASE TRANSLATION SYSTEM [5]: CELLULOSE-POLY U·70S·POLYPHE-tRNA, PHE-tRNA, EF-T, EF-G, GTP					
I	TRANSFER INTO COLUMNS ①	②	③	④	⑤	⑥
II	WASHING BY BUFFER WITH Mg^{2+}					
III	+ PHE-tRNA, + EF-T, + GTP					
IV	WASHING BY BUFFER WITH Mg^{2+}					
V	NONE	+ EF-G	+ GTP	+ GMPPCP	+ EF-G, + GTP	+ EF-G, + GMPPCP
VI	WASHING BY BUFFER WITH Mg^{2+}					
VII	+ PUROMYCIN (polyphe-PM is released from ribosomes and adsorbed partly on the resin)					
VIII	WASHING BY BUFFER WITH Mg^{2+} (non-adsorbed polyphe-PM is washed off)					
IX	WASHING BY BUFFER WITHOUT Mg^{2+} (ribosomes and polyphe-tRNA are washed off)					
X	WASHING BY 0.5 N KOH IN ETHANOL (resin-adsorbed polyphe-PM is washed off)					

Fig.1. Scheme of the experiment on translocation in ribosomes bound in a polyU-cellulose column.

Table 1
Puromycin competence of [^{14}C]polyphenylalanyl-tRNA in cellulose-polyU-bound ribosomes

Column No. (see scheme, fig.1)	Components passed through the column (Stage V in fig.1)	Total [^{14}C]phenylalanine polymerized, pmoles per column	Puromycin-released [^{14}C]phenylalanine polymerized	
			pmol per column	%% of the total
	Set 1:			
1	None	32.5	9.7	30
2	EF-G	31.2	10.4	33
3	GTP	27.8	9.5	34
4	GMPPCP	33.5	11.6	35
	Set 2:			
1	None	21.5	6.5	30
2	EF-G	22.1	8.1	37
5	EF-G + GTP	21.9	14.2	65
6	EF-G + GMPPCP	22.8	14.9	65

the table that in the experiment presented, the initial portion of the ribosomes competent to puromycin, i.e. those in the post-translocation state, is about 30–35%. (This portion usually varied from 20% to 50% in analogous experiments; such relatively high values can be explained either by incomplete enzymatic binding and incorporation of phenylalanyl-tRNA at the preceding stage, or by spontaneous non-enzymatic translocation [6,10] in the course of column washing). The passing through the column of either EF-G without GTP or only GTP or only GMPPCP does not increase the portion of post-translocated ribosomes (table 1, Set 1). Translocation is induced by passing of EF-G together with GTP: the portion of ribosomes competent to puromycin increases up to 65%. The main result of the experiment is the fact that the passing of EF-G with the non-cleavable analogue of GTP, GMPPCP, produces the same translocation as does EF-G with GTP (table 1, Set 2).

The result of the experiment seems to evidence unambiguously that GTP cleavage is not necessary for EF-G-induced translocation. The attachment-detachment of EF-G seems rather a more probable driving mechanism of translocation in the ribosome.

In their experiments Vazquez's group [3] did not succeed in producing translocation by substitution of GMPPCP for GTP. If the experiments of both Vazquez et al. and ours are taken to be correct, then the difference in the results can be explained only by the difference in experimental conditions: competence to puromycin does not appear when EF-G with GMPPCP are still present in the medium and in the ribosomes [3], but it appears when EF-G with GMPPCP is washed off (fig.1 and table 1). From this it follows that; 1) either the attachment of EF-G with GMPPCP to the ribosome is sufficient to perform translocation, but the bound EF-G inhibits the puromycin reaction; or 2) translocation is performed (completed) by the detachment of EF-G.

Kaziro et al. [4] succeeded in producing translocation when GMPPCP was substituted for GTP, despite the presence of EF-G with GMPPCP in the medium. If the experiments cited are also correct, it is only to be assumed that incubation of pretranslocated ribosomes with EF-G and GMPPCP at 30°C resulted in the attachment of EF-G, while the subsequent transfer of the incubation mixture into conditions at 0°C [4] led to dissociation of EF-G from the ribosome.

On the basis of the available data it can be thought that the attachment–detachment of a protein factor, and not GTP cleavage by itself, is a general principle of functioning of other translation factors as well, such as initiation factor IF-2 [11–13], elongation factor EF-T_u [14] and termination factors RF [15].

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